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Skin-Cancer Protective Effect of *Ziziphus Spina-christi* Leaf Extract: *In vitro and in Vivo* Models

Omar F. Khabour[1,*], Karem H. Alzoubi[2], Ahmad S. Alkofahi[3], Rafat M. Al-Awad[1]

[1] *Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Jordan University of Science and Technology, Irbid, Jordan;* [2]*Department of Clinical Pharmacy, Jordan University of Science and Technology, Irbid, Jordan;* [3]*Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, 22110, Jordan.*

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Abstract

Cancer prevention includes approaches that can reduce the risk of developing cancer, such as maintaining a healthy diet and avoiding exposure to mutagenic agents. Plants with antimutagenic properties can be valuable as cancer-protective agents. This study aims to investigate the antimutagenic activity and cancer-protective effects of *Ziziphus spina-christi* using *in vitro* and *in vivo* human and animal models, respectively.

The antimutagenic activity of *Ziziphus spina-christi* leaf extract was examined in cultured human lymphocytes using sister-chromatid exchanges (SCEs) and 8-OHdG assays. In addition, the cancer-protective effect of the extract was investigated using a skin painting assay and7,12-dimethylbenz(a) anthracene (DMBA)induced cancer of Balb/c mice. Animals were treated with DMBA for 20 weeks with concurrent consumption of *Ziziphus spina-christi* leaf extract 100mg/kg daily, 5 days/week. The *Ziziphus spina-christi* leaf extract significantly reduced the levels of SCEs and the 8-OHdG levels in human lymphocytes in a dose-response manner ($P<0.01$). In addition, leaf extract significantly reduced the incidence of skin tumors, tumor sizes, and frequency of DMBA-induced papilloma ($P<0.05$). It also delayed the onset of tumor development compared to the DMBA group ($P<0.05$). Finally, animals treated with *Ziziphus spina-christi* leaf extract had lower body weight compared to the control group ($P>0.05$). In conclusion, the experimental investigation demonstrated the beneficial properties of *Ziziphus spina-christi* as an anticancer agent.

Keywords: plant extract, *Ziziphus*, carcinoma, skin cancer, cancer prevention.

1. Introduction

Skin cancer (SC)is a common disease affecting a large number of individuals in the world (Ahmed et al., 2020). The three most common types of SC are melanoma, basal cell carcinoma, and cutaneous squamous cell carcinoma (Collins et al., 2019). Although various therapies have been applied to cure cancer, researchers have been searching for more potent and simultaneously safer medications to beat the disease (Lalotra et al., 2020). Natural compounds such as plant products have been utilized as antitumor agents for different types of cancers (Sajadimajd et al., 2020; Sauter, 2020). In addition, many plant products have shown antimutagenic activities and thus can be used as protective agents against the development of cancer (Alzoubi et al., 2021; Fahmy et al., 2022; Nageen et al., 2021).

Among the promising medicinal plants is *Ziziphus spina-christi* which belongs to the plant family *Rhamnaceae* (Abdulrahman et al., 2022).More than 40 species have been classified within this family, with a wide range of medicinal uses including the management of chronic diseases, infectious diseases and malignancies (Al Omar et al., 2022). *Ziziphus spina-christi* is an evergreen plant that is native to north/tropical Africa, and South/West Asia (Abdulrahman et al., 2022; Albalawi, 2021). Various studies have revealed the valuable components of *Ziziphus spina-christi* plant such as botulin, spinanine, tanines, sterols, rutin, quercetin derivatives, triterpenoids, sapogenins, and saponins (Bozicevic et al., 2017; Hussein, 2019; Soliman et al., 2019). *Ziziphus spina-christi* plant extracts have shown a potent function against cancer cell lines (El-Maksoud et al., 2021; Fahmy et al., 2022; Farmani et al., 2016; Jafarian et al., 2014). Additionally, the plant leaf extract has been reported to be effective in fighting bacterial and viral infections as well as reducing diarrhea symptoms and diabetes activity (Abdulrahman et al., 2022; Albalawi, 2021; Hussein, 2019; Zandiehvakili and Khadivi, 2021).

In the current study, the antimutagenic activity of *Ziziphus spina-christi* leaf extract was examined in cultured human lymphocytes using sister-chromatid exchanges (SCEs) and 8-hydroxy-2' -deoxyguanosine (8-OHdG) assays. In addition, the cancer-protective effect of the extract was investigated using mouse skin painting assay. The 8-OHdG assay is widely used to examine the level of DNA damage caused by oxidative stress in living cells. The SCEs assay is used as an indicator of DNA strand damage induced by various mutagenic agents. Finally, the skin painting assay is a valid and widely used model for examining the carcinogenic potential of

* Corresponding author. e-mail: khabour@just.edu.jo.

chemical agents. In addition, the model has also been used to investigate treatments that can be useful as protective agents against the development of cancer.

2. Materials and Methods

2.1. Animals

Young (7-8 weeks old) Balb/c mice were purchased from the Animal Care Facility of Jordan University of Science and Technology (JUST). Animals were housed in standard mice cages (4-6 animals per cage) and underwent 14 days of acclimatization to experimental conditions prior to the start of the experiments (Alzoubi et al., 2020).The animals were kept in optimal experimental conditions that included temperature (24±1°C), humidity (45±5)%, light/dark cycle (12h/12h), and food/water (*ad libitum*). Animals were shaved in the interscapular region 3 days before the start of the experiment. The animal part of the study was approved by the Animal Care and Use Committee at JUST.

2.2. Ziziphus spina-christi leaf *extract*

Ziziphus spina-christi was collected from Jordan, Irbid region under the supervision of Professor Jamal Lahaam, a botanist from Yarmouk University (Irbid, Jordan). The leaves of the plant were dried, stored, and a gummy extract was prepared as previously described (Alkofahi et al., 2017). In brief, after drying, *Ziziphus spina-christi* leaves were mechanically stranded using a 2 mm mesh machine (Wiley grinder, Homas Scientific, USA). Stranded leaves were percolated in ethanol (80%) and then subjected to vacuum evaporation at 40 °C. The extracts were stored in aliquots at -30 °C until used (Alkofahi et al., 2016).

2.3. Skin paint assay

Animals were divided into 3 homogeneous groups of 15 mice each. Animals were assigned into groups according to their body weight using Xybion PATH/TOX software, Cedar Knolls, NJ, USA. The three groups were: the control group (treated with vehicle), DMBA group (100 ug of 7,12-dimethylbenz(a) anthracene, Sigma-Aldrich, Saint Louis, MO, USA) and DMBA/plant extract group (Alzoubi et al., 2021). DMBA, the tumor induction agent, was dissolved in acetone and immediately applied to the interscapular skin of animals using micropipettes. Animals in the DMBA/plant extract group were treated with DMBA as in group 2 plus *Ziziphus spina-christi* leaf extracts (administered using oral gavage, dose = 100 mg/kg). DMBA and the plant extract were administered daily except for Friday and Saturday of each week during the 20-week experimental period (Li and Brakebusch, 2021). The general health of the animals was checked regularly in each working day during the study period. Regarding tumor development, the skin of the animals was examined weekly. Papillomas with diameters of 2mm or larger were recorded (Roemer et al., 2010). In addition, the following measures were recorded: tumor incidence (% of tumor-bearing mice), multiplicity (% of tumors/mice), and tumor onset(Ghosh et al., 2015; Gopalakrishnan et al., 2019).

2.4. Blood Cultures

Blood was obtained from 5 health donors in heparin vacutainer tubes. The criteria for the selection of blood donors were as previously described (Hendawi et al., 2022; Tarboush et al., 2022). For each 1mL blood culture, 9 mL of PB-Max media (Thermo Fisher Scientific, MA, USA) were used. Blood cultures were maintained at 37°C in a humid CO2 incubator as described previously (Rababa'h et al., 2019). Cultures assigned to SCEs were treated with 5-bromodeoxyuridine (BrdU, 25 µg/mL final concentration, Sigma-Aldrich, USA) to obtain differential staining of sister-chromatids (Al-Eitan et al., 2020).

2.5. In vitro sister-chromatid exchanges (SCEs) assay

Cultures assigned for SCEs were treated with leaf extracts (10, 100, and 500 ug/mL final concentration) in the last 24 hours of the culture period. Lymphocytes at metaphase were harvested by treating the cultures with colcemid (10 ug/mL final concentration). The cultures were then subjected to a hypotonic solution of KCl and then the cells were collected by centrifugation and fixed in methanol/acetic acid (3/1 respectively). Fixed cells were spotted on microscopic glass slides by dropping and then stained using the Fluorescence Plus Giemsa (FPG) method as previously described (Khabour et al., 2013). At least 250 metaphase II cells were scored for each treatment (Alqudah et al., 2018).

2.6. The 8-OHdG assay

At the end of the 72 hours incubation period, blood cultures assigned for 8-OHdG assay were centrifuged at 300xg, and the culture media was removed. The cells were then washed 5 times using RBMI media. The cells were then treated in RBMI media with different concentrations of leaf extracts (10, 100, and 500 in ug/mL final concentration) for 6 hours (Daradka et al., 2018). The cell suspension was then centrifuged at 300 xg, and 8-OHdG in the supernatant was measured using a commercial ELISA assay obtained from Abcam (Cambridge, UK). ELISA plates were read at 405 nm using the ELx800 ELISA plate reader (BIO-TEK, USA) as previously described (Azab et al., 2017). A total of 8 replicates were used for each concentration.

2.7. Statistical analysis

Data were analyzed using GraphPad Prism Software (version 5, La Jolle, CA, USA). Groups were compared using one-way ANOVA followed by Tukey's post hoc test. A $p<0.05$ indicates statistical significance.

3. Results

Ziziphus spina-christi leaf extracts were tested for antimutagenic potential using SCEs and 8-OHdG assays in cultured human lymphocytes. Figure 1 shows SCEs levels in lymphocytes treated with different concentrations (10, 100, and 500 ug/mL) of the plant extract. *Ziziphus spina-christi* leaf extracts at 100 ug/mL and 500 ug/mL induced a significant decrease in the frequency of SCEs compared to the control group ($P<0.01$). However, *Ziziphus spina-christi* leaf extracts at 10 ug/mL did not affect the frequency of SCEs compared to the control group ($P<0.05$). The magnitude of the decrease in the frequency of SCEs resulting from the 100 ug/mL concentration is similar to that observed at 500 ug/mL This indicates a saturation effect at the 100 ug/mL concentration. Figure 2 shows the levels of 8-OHdG in cultures treated with different concentrations (10, 100, and 500 ug/mL) of the

leaf extract. A significant decrease in 8-OHdG levels was observed for all examined concentrations of *Ziziphus spina-christi* leaf extract. The magnitudes of the decrease in 8-OHdG caused by 100 ug/mL and 500 ug/mL were significantly higher than that induced by the 10 ug/mL concentration (P<0.01). In addition, both concentrations of100 ug/mL and 500 ug/mL induced a similar reduction in the levels of the 8-OHdG marker, indicating a saturation effect. Results of sister-chromatid exchange and 8-OHdG assays indicate the antimutagenic effects of the *Ziziphus spina-christi* plant extract.

The protective effect of the leaf extract against cancer development was examined using the mouse skin painting assay (Figure 3). The results showed that *Ziziphus spina-christi* leaf extract significantly lowered the incidence of skin tumors, tumor sizes, and frequency of papilloma (P<0.05) induced by treating animal skin with DMBA (Figures 3C, 3E, and 3F respectively). Additionally, the *Ziziphus spina-christi* plant extract significantly retarded the onset of skin tumors (P<0.05) (Figure 3D). On the other hand, animals treated with *Ziziphus spina-christi* leaf extract showed marked decreases in body weight when compared with the control group (Figure 3A, P>0.05). Finally, leaf extract did not affect the number of tumors/animal (Figure 3B, P>0.05).

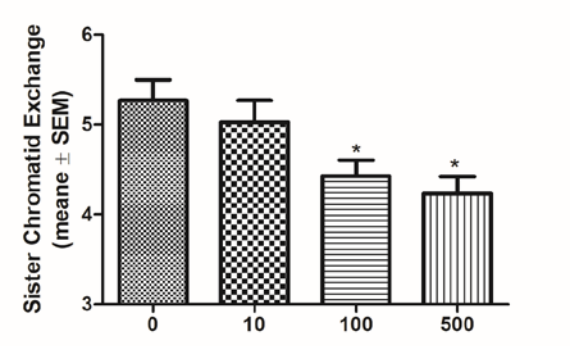

Figure 1. Antimutagenic activity of *Ziziphus spina-christi* leaf extract using sister-chromatid exchanges (SCEs) assay. *Ziziphus spina-christi* leaf extract significantly reduced levels of SCEs in cultured human lymphocytes at 100 µg/mL and 500 µg/mL concentrations (* indicates P<0.01).

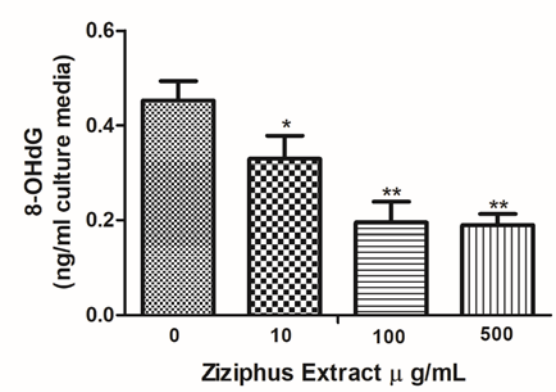

Figure 2. Antimutagenic activity of *Ziziphus spina-christi* leaf extract using 8-OHdG assay.*Ziziphus spina-christi* leaf extract significantly reduced levels of 8-OHdG in cultured human lymphocytes at 10 µg/mL, 100 µg/mL and 500 µg/mL concentrations (* indicates P<0.05, ** indicates P<0.01).

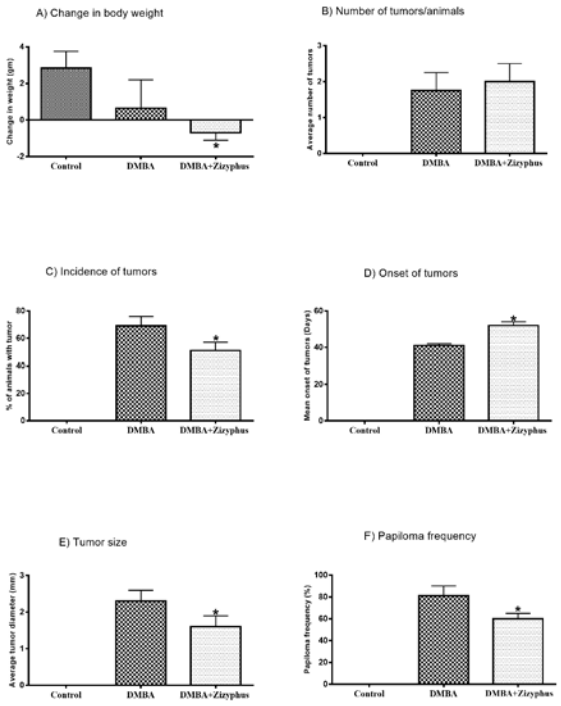

Figure 3. Cancer-prevention effect of *Ziziphus spina-christi* leaf extract.

Mouse skin-painting assay was used. *Ziziphus spina-christi* leaf extract was administered using oral gavage, once daily (100mg/kg) for 5 days/week until the end of the experiment. Skin tumor was induced using DMBA. *Ziziphus spina-christi* leaf extract significantly reduced the incidence of skin tumors (C), tumor sizes (E), and frequency of papilloma (F). *Ziziphus spina-christi* plant extract significantly delayed the onset of skin tumors (D) and lowered the body weight of animals (A). Finally, leaf extract did not affect the number of tumors/animal (B). * indicates P<0.05.

4. Discussion

Cancer remains among the leading causes of death worldwide despite the remarkable improvement in the treatment of most malignancies(Torre et al., 2016). Therefore, adopting a preventive strategy for cancer can reduce the burden and death associated with this disease. Natural products with antimutagenic and antioxidant potential are good candidates that can help in cancer prevention(Sanders et al., 2016).

In the current study, the antimutagenic activity and cancer-prevention effects of the medicinal plant *Ziziphus spina-christi* were examined. The results showed a strong antimutagenic activity of *Ziziphus spina-christi* in vitro in cultured human lymphocytes. In addition, consumption of *Ziziphus spina-christi* leaf extract significantly reduced the carcinogenicity of DMBA-induced skin cancer.

The antimutagenicity of *Ziziphus spina-christi* leaf extract was demonstrated in the current study using SCEs and the 8-OHdG assays. The antimutagenic effects of *Ziziphus spina-christi* leaf extract are dose-dependent (P<0.01). SCEs in mitotic cells reflect damage to DNA strands that are usually corrected using a recombination DNA repair system that utilizes the unaltered homologous

DNA to fix the damaged strand. The 8-OHdG biomarker reflects DNA damage induced by free radicals generated within cells during cellular respiration and by chemical/physical agents. Therefore, *Ziziphus spina-christi* leaf extract appears to protect cells from a spectrum of DNA damage through single/multiple mechanisms. Among these mechanisms is the potent antioxidant property of *Ziziphus spina-christi* leaf extract which has been shown to neutralize more than 90% of cellular-induced oxidative stress (Almeer et al., 2018; Hosny et al., 2022; Zandiehvakili and Khadivi, 2021). Among the known antioxidants of *Ziziphus spina-christi* leaf extract are glycosides, phenols, terpenoids, cyclopeptide alkaloids, and flavonoids (Elghaffar et al., 2022; Sakna et al., 2019). In addition, consumption of the leaf extract has been shown to reduce oxidative stress and improve recovery from brain injury via modulatingaquaporin-4 and TLR4/NF-κB/TNF-α signaling pathways (Safdari et al., 2021). Using a rat animal model, *Ziziphus spina-christi* leaf extract has been reported to attenuate mercuric chloride-induced liver and testicular injury via normalizing oxidative stress (Almeer et al., 2020; Ramadan et al., 2021). The antioxidative status of rabbits fed a diet supplemented with *Ziziphus spina-christi* leaves showed significant improvements in blood total antioxidative capacity and glutathione peroxidase, catalase and superoxide dismutase activities (Abduljawad, 2020). Therefore, using different animal models, *Ziziphus spina-christi* showed potent activity against the body's oxidative stress.

In the current study, the cancer-protective activity of *Ziziphus spina-christi* leaf extract was demonstrated using the mouse skin painting assay. A significant reduction in several measures of tumor development was observed in animals that consumed *Ziziphus spina-christi* leaf extract. These include the incidence of tumors, tumor sizes, and frequency of induced papilloma by the carcinogen. In addition, tumor onset was significantly delayed in the animals that received *Ziziphus spina-christi* leaf extract. Consistent with the current study, methanolic *Ziziphus spina-christi* leaf extract has been reported to protect Wistar rats from hepatocellular carcinoma induced by diethyl nitrosamine (El-Din et al., 2019). Similarly, *Ziziphus spina-christi* fruit extract has been shown to protect animals from azoxymethane-induced colon cancer (Guizani et al., 2013). The anticancer effects of *Ziziphus spina-christi* leaf extract were attributed to its high content of polyphenols, flavonoids, betulin derivatives, and cyclopeptide alkaloids compounds (Abdulrahman et al., 2022; Soliman et al., 2019).

Several mechanisms may mediate the cancer-protective effects of *Ziziphus spina-christi* leaf extract. *Ziziphus spina-christi* leaf extract contains numerous bioactive compounds with potent anticancer activity reported using human and animal cancer cell lines (Abdulrahman et al., 2022; El-Maksoud et al., 2021; Jafarian et al., 2014; Sakna et al., 2019; Soliman et al., 2019). For example, *Ziziphus spina-christi* leaf extract showed strong activity against human MCF7breast cancer cells with an IC50 of 40.25 µg/mL(Hosny et al., 2022). Another mechanism by which *Ziziphus spina-christi* leaf extract can prevent the development of cancer is via promoting apoptosis in cancer cells by activating the Bax-independent apoptosis pathway (Farmani et al., 2016; Ghaffari et al., 2021). In addition, *Ziziphus spina-christi* leaf extract can significantly reduce tissue inflammation, which is a predisposing factor for the development of cancer (Alajmi et al., 2019; Kadioglu et al., 2016). A human study showed the efficacy of *Ziziphus spina-christi* leaf extract in protecting against skin rashes associated with anticancer medications (Alzahrani et al., 2019). Finally, the antimutagenic activity of *Ziziphus spina-christi* leaf extracts reported in the current study may play an important role in protecting animals from skin cancer.

Animals that consumed *Ziziphus spina-christi* leaf extract showed a notable reduction in body weight. This observation is consistent with previous studies using other animal models. For example, a reduction in the body weight of obese rats has been reported after the consumption of Ziziphus tea (El Seedy et al., 2021).The mechanism by which *Ziziphus spina-christi* reduces body weight may involve promoting fatty acid oxidationin mitochondria and reducing adipokine secretions (Yagoub et al., 2021). However, in a goat bread, adding *Ziziphus spina-christi* leaves to the diet enhanced food intake and increased body weight (Ali et al., 2019). Therefore, further studies are needed to confirm the current findings on the effect of *Ziziphus spina-christi* leaf extract on animals' body weight.

Among the limitations of the current study is that the crude extract of the leaves was used. Fractionation of the crude extract and identification of active antimutagenic and anticancer compounds is recommended. In addition, future *in vitro* studies using skin cancer cell lines are needed to confirm the current findings.

Conclusion

Ziziphus spina-christi leaf extract is beneficial for cancer prevention by reducing the mutagenicity of cancer-inducing agents.

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References

Abduljawad, S.H. 2020. Digestive Fermentation, Antioxidant Status, and Haemato-Biochemical Indices of Growing Rabbits Fed on Diets Supplemented with Ziziphus spina-christi Leaf. *J Nutr Metab*. **2020**:9046862.

Abdulrahman, M.D., A.M. Zakariya, H.A. Hama, S.W. Hamad, S.S. Al-Rawi, S.W. Bradosty, and A.H. Ibrahim. 2022. Ethnopharmacology, Biological Evaluation, and Chemical Composition of Ziziphus spina-christi (L.) Desf.: A Review. *Adv Pharmacol Pharm Sci*. **2022**:4495688.

Ahmed, B., M.I. Qadir, and S. Ghafoor. 2020. Malignant Melanoma: Skin Cancer-Diagnosis, Prevention, and Treatment. *Crit Rev Eukaryot Gene Expr*. **30**(**4**):291-297.

Al-Eitan, L.N., K.H. Alzoubi, L.I. Al-Smadi, and O.F. Khabour. 2020. Vitamin E protects against cisplatin-induced genotoxicity in human lymphocytes. *Toxicol In Vitro*. **62**:104672.

Al Omar, R., R. Micklewright, K. Masud, T. Naz, S. Vemulpad, and J. Jamie. 2022. The genus Alphitonia Reissek ex Endl. (Rhamnaceae): A review of its customary uses, phytochemistry and biological activities. *J Ethnopharmacol*. **294**:115168.

Alajmi, R.A., W.A. Al-Megrin, D. Metwally, H. Al-Subaie, N. Altamrah, A.M. Barakat, A.E. Abdel Moneim, T.T. Al-Otaibi, and M. El-Khadragy. 2019. Anti-Toxoplasma activity of silver nanoparticles green synthesized with Phoenix dactylifera and Ziziphus spina-christi extracts which inhibits inflammation through liver regulation of cytokines in Balb/c mice. *Biosci Rep*. **39**(**5**):BSR20190379.

Albalawi, A.E. 2021. Antileishmanial Activity of Ziziphus spina-christi Leaves Extract and Its Possible Cellular Mechanisms. *Microorganisms*. **9**(**10**):2113.

Ali, A., F. Tegegne, B. Asmare, and Z. Mekuriaw. 2019. On farm evaluation of sun-dried Ziziphus spina-christi leaves substitution for natural pasture hay on feed intake and body weight change of Bati goat breeds in Ethiopia. *Trop Anim Health Prod*. **51**(**2**):457-463.

Alkofahi, A.S., K.K. Abdul-Razzak, K.H. Alzoubi, and O.F. Khabour. 2017. Screening of the Anti-hyperglycemic activity of some medicinal plants of Jordan. *Pak J Pharm Sci*. **30**(**3**):907-913.

Alkofahi, A.S., K.H. Alzoubi, O.F. Khabour, and N.M. Mhaidat. 2016. Screening of selected medicinal plants from Jordan for their protective properties against oxidative DNA damage. *Ind Crops Prod*. **88**:106-111.

Almeer, R.S., G. Albasher, R.B. Kassab, S.R. Ibrahim, F. Alotibi, S. Alarifi, D. Ali, S. Alkahtani, and A.E. Abdel Moneim. 2020. Ziziphus spina-christi leaf extract attenuates mercury chloride-induced testicular dysfunction in rats. *Environ Sci Pollut Res Int*. **27**(**3**):3401-3412.

Almeer, R.S., M.F. El-Khadragy, S. Abdelhabib, and A.E. Abdel Moneim. 2018. Ziziphus spina-christi leaf extract ameliorates schistosomiasis liver granuloma, fibrosis, and oxidative stress through downregulation of fibrinogenic signaling in mice. *PLoS One*. **13**(**10**):e0204923.

Alqudah, M.A.Y., F.Y. Al-Ashwal, K.H. Alzoubi, M. Alkhatatbeh, and O. Khabour. 2018. Vitamin E protects human lymphocytes from genotoxicity induced by oxaliplatin. *Drug Chem Toxicol*. **41**(**3**):281-286.

Alzahrani, A.M., A.A. Alzahrani, and A.A. Alsharm. 2019. The Use of Ziziphus spina-christi Extract in Treating Erlotinib (Tarceva®) Associated Rash: A Case Report. *Case Rep Oncol*. **12**(**3**):909-912.

Alzoubi, K.H., L. Abdel-Hafiz, O.F. Khabour, T. El-Elimat, M.A. Alzubi, and F.Q. Alali. 2020. Evaluation of the Effect of Hypericum triquetrifolium Turra on Memory Impairment Induced by Chronic Psychosocial Stress in Rats: Role of BDNF. *Drug Des Devel Ther*. **14**:5299-5314.

Alzoubi, K.H., O.F. Khabour, A.S. Alkofahi, N.M. Mhaidat, and A.A. Abu-Siniyeh. 2021. Anticancer and antimutagenic activity of Silybum marianum L. and Eucalyptus camaldulensis Dehnh. against skin cancer induced by DMBA: In vitro and in vivo models. *Pak J Pharm Sci*. **34**(**3**).

Azab, M., O.F. Khabour, K.H. Alzoubi, H. Hawamdeh, M. Quttina, and L. Nassar. 2017. Assessment of genotoxicity of pyrethrin in cultured human lymphocytes. *Drug Chem Toxicol*. **40**(**3**):251-255.

Bozicevic, A., M. De Mieri, A. Di Benedetto, F. Gafner, and M. Hamburger. 2017. Dammarane-type saponins from leaves of Ziziphus spina-christi. *Phytochem*. **138**:134-144.

Collins, L., A. Quinn, and T. Stasko. 2019. Skin Cancer and Immunosuppression. *Dermatol Clin*. **37**(**1**):83-94.

Daradka, H.M., O.F. Khabour, and M.K. Alotaibi. 2018. Potent antioxidative DNA damage of selected Saudi medicinal plants in cultured human lymphocytes. *Pak J Pharm Sci*. **31**(**4(Supplementary)**):1511-1517.

El-Din, M.S., A.M. Taha, A.A. Sayed, and A.M. Salem. 2019. Ziziphus spina-christi leaves methanolic extract alleviates diethylnitrosamine-induced hepatocellular carcinoma in rats. *Biochem Cell Biol*. **97**(**4**):437-445.

El-Maksoud, A.A.A., A.I.A. Makhlouf, A.B. Altemimi, I.H.A. El-Ghany, A. Nassrallah, F. Cacciola, and T.G. Abedelmaksoud. 2021. Nano Milk Protein-Mucilage Complexes: Characterization and Anticancer Effect. *Molecules*. **26**(**21**).

El Seedy, G.M., E.S. El-Shafey, and E.S. Elsherbiny. 2021. Ziziphus spina-christi (L.) fortified with Camellia sinensis mediates apoptosis, Notch-1 signaling, and mitigates obesity-induced non-alcoholic fatty liver. *J Food Biochem*:e13849.

Elghaffar, R.Y.A., B.H. Amin, A.H. Hashem, and A.E. Sehim. 2022. Promising Endophytic Alternaria alternata from Leaves of Ziziphus spina-christi: Phytochemical Analyses, Antimicrobial and Antioxidant Activities. *Appl Biochem Biotechnol*. **194**(**9**):3984-4001.

Fahmy, M.A., A.A. Farghaly, E.E. Hassan, E.M. Hassan, Z.M. Hassan, K. Mahmoud, and E.A. Omara. 2022. Evaluation of the Anti-Cancer/Anti-Mutagenic Efficiency of Lavandula officinalis Essential Oil. *Asian Pac J Cancer Prev*. **23**(**4**):1215-1222.

Farmani, F., M. Moein, A. Amanzadeh, H.M. Kandelous, Z. Ehsanpour, and M. Salimi. 2016. Antiproliferative Evaluation and Apoptosis Induction in MCF- 7 Cells by Ziziphus spina christi Leaf Extracts. *Asian Pac J Cancer Prev*. **17**(**1**):315-321.

Ghaffari, K., R. Ahmadi, B. Saberi, and P. Moulavi. 2021. Anti-proliferative effects of Ziziphus spina-christi and Phlomis russeliana leaf extracts on HEK293 and MCF-7 Cell Lines and Evaluation of Bax and Bcl-2 Genes Expression Level in MCF-7 Cells. *Asian Pac J Cancer Prev*. **22**(**S1**):81-87.

Ghosh, S., S. Sikdar, A. Mukherjee, and A.R. Khuda-Bukhsh. 2015. Evaluation of chemopreventive potentials of ethanolic extract of Ruta graveolens against A375 skin melanoma cells in vitro and induced skin cancer in mice in vivo. *J Integr Med*. **13**(**1**):34-44.

Gopalakrishnan, T., S. Ganapathy, V. Veeran, and N. Namasivayam. 2019. Preventive effect of D-carvone during DMBA induced mouse skin tumorigenesis by modulating xenobiotic metabolism and induction of apoptotic events. *Biomed Pharmacother*. **111**:178-187.

Guizani, N., M.I. Waly, V. Singh, and M.S. Rahman. 2013. Nabag (Zizyphus spina-christi) extract prevents aberrant crypt foci development in colons of azoxymethane-treated rats by abrogating oxidative stress and inducing apoptosis. *Asian Pac J Cancer Prev*. **14**(**9**):5031-5035.

Hendawi, E.K., O.F. Khabour, L.N. Al-Eitan, and K.H. Alzoubi. 2022. Reduction of Genotoxicity of Carbamazepine to Human Lymphocytes by Pre-Treatment with Vitamin B12. *Curr Mol Pharmacol*.

Hosny, M., A.S. Eltaweil, M. Mostafa, Y.A. El-Badry, E.E. Hussein, A.M. Omer, and M. Fawzy. 2022. Facile Synthesis of Gold Nanoparticles for Anticancer, Antioxidant Applications, and Photocatalytic Degradation of Toxic Organic Pollutants. *ACS Omega*. **7**(**3**):3121-3133.

Hussein, A.S. 2019. Ziziphus spina-christi: Analysis of bioactivities and chemical composition. *In* Wild Fruits: Composition, **Nutritional Value and Products**. Springer. 175-197.

Jafarian, A., B. Zolfaghari, and K. Shirani. 2014. Cytotoxicity of different extracts of arial parts of Ziziphus spina-christi on Hela and MDA-MB-468 tumor cells. *Adv Biomed Res*. **3**:38.

Kadioglu, O., S. Jacob, S. Bohnert, J. Naß, M.E. Saeed, H. Khalid, I. Merfort, E. Thines, T. Pommerening, and T. Efferth. 2016. Evaluating ancient Egyptian prescriptions today: Anti-inflammatory activity of Ziziphus spina-christi. *Phytomed*. **23**(**3**):293-306.

Khabour, O.F., O.A. Soudah, and M.H. Aaysh. 2013. Genotoxicity assessment in iron deficiency anemia patients using sister chromatid exchanges and chromosomal aberrations assays. *Mutat Res*. **750**(**1-2**):72-76.

Lalotra, A.S., V. Singh, B. Khurana, S. Agrawal, S. Shrestha, and D. Arora. 2020. A Comprehensive Review on Nanotechnology-Based Innovations in Topical Drug Delivery for the Treatment of Skin Cancer. *Curr Pharm Des*. **26**(**44**):5720-5731.

Li, H., and C. Brakebusch. 2021. Analyzing skin tumor development in mice by the DMBA/TPA model. *Methods Cell Biol*. **163**:113-121.

Nageen, B., A. Rasul, G. Hussain, M.A. Shah, H. Anwar, S.M. Hussain, M.S. Uddin, I. Sarfraz, A. Riaz, and Z. Selamoglu. 2021. Jaceosidin: A Natural Flavone with Versatile Pharmacological and Biological Activities. *Curr Pharm Des*. **27**(**4**):456-466.

Rababa'h, A.M., O.F. Khabour, K.H. Alzoubi, D. Al-Momani, and M. Ababneh. 2019. Assessment of Genotoxicity of Levosimendan in Human Cultured Lymphocytes. *Curr Mol Pharmacol*. **12**(**2**):160-165.

Ramadan, S.S., R.S. Almeer, S. Alkahtani, S. Alarifi, G. Albasher, and A.E. Abdel Moneim. 2021. Ziziphus spina-christi leaf extract attenuates mercuric chloride-induced liver injury in male rats via inhibition of oxidative damage. *Environ Sci Pollut Res Int*. **28**(**14**):17482-17494.

Roemer, E., T.H. Ottmueller, H.J. Urban, and C. Baillet-Mignard. 2010. SKH-1 mouse skin painting: a short-term assay to evaluate the tumorigenic activity of cigarette smoke condensate. *Toxicol Lett*. **192**(**2**):155-161.

Safdari, M.R., F. Shakeri, A. Mohammadi, B. Bibak, P. Alesheikh, T. Jamialahmadi, T. Sathyapalan, and A. Sahebkar. 2021. Role of Herbal Medicines in the Management of Brain Injury. *Adv Exp Med Biol*. **1328**:287-305.

Sajadimajd, S., R. Bahramsoltani, A. Iranpanah, J. Kumar Patra, G. Das, S. Gouda, R. Rahimi, E. Rezaeiamiri, H. Cao, F. Giampieri, M. Battino, R. Tundis, M.G. Campos, M.H. Farzaei, and J. Xiao. 2020. Advances on Natural Polyphenols as Anticancer Agents for Skin Cancer. *Pharmacol Res*. **151**:104584.

Sakna, S.T., A. Mocan, H.N. Sultani, N.M. El-Fiky, L.A. Wessjohann, and M.A. Farag. 2019. Metabolites profiling of Ziziphus leaf taxa via UHPLC/PDA/ESI-MS in relation to their biological activities. *Food Chem*. **293**:233-246.

Sanders, K., Z. Moran, Z. Shi, R. Paul, and H. Greenlee. 2016. Natural Products for Cancer Prevention: Clinical Update 2016. *Semin Oncol Nurs*. **32**(**3**):215-240.

Sauter, E.R. 2020. Cancer prevention and treatment using combination therapy with natural compounds. *Expert Rev Clin Pharmacol*. **13**(**3**):265-285.

Soliman, S., A.M. Hamoda, A.A. El-Shorbagi, and A.A. El-Keblawy. 2019. Novel betulin derivative is responsible for the anticancer folk use of Ziziphus spina-christi from the hot environmental habitat of UAE. *J Ethnopharmacol*. **231**:403-408.

Tarboush, N.A., D.H. Almomani, O.F. Khabour, and M.I. Azzam. 2022. Genotoxicity of Glyphosate on Cultured Human Lymphocytes. *Int J Toxicol*. **41**(**2**):126-131.

Torre, L.A., R.L. Siegel, E.M. Ward, and A. Jemal. 2016. Global Cancer Incidence and Mortality Rates and Trends--An Update. *Cancer Epidemiol Biomarkers Prev*. **25**(**1**):16-27.

Yagoub, A.E.A., G.M. Alshammari, P. Subash-Babu, M.A.A. Mohammed, M.A. Yahya, and A.I. Alhosain. 2021. Synthesis of Ziziphus spina-christi (Jujube) Root Methanol Extract Loaded Functionalized Silver Nanoparticle (ZS-Ag-NPs); Physiochemical Characterization and Effect of ZS-Ag-NPs on Adipocyte Maturation, Adipokine and Vascular Smooth Muscle Cell Interaction. *Nanomaterials (Basel)*. **11**(**10**): 2563.

Zandiehvakili, G., and A. Khadivi. 2021. Identification of the promising Ziziphus spina-christi (L.) Willd. genotypes using pomological and chemical proprieties. *Food Sci Nutr*. **9**(**10**):5698-5711.